

DIABLO Polyclonal Antibody

Catalog NumberPA5-19974

Product data sheet

Details		Species Reactivity	
Size	100 µg	Species reactivity	Human, Mouse, Rat
Host/Isotope	Rabbit / IgG	Tested Applications	
Class	Polyclonal	Immunohistochemistry (IHC)	5 µg/mL
Type	Antibody	Immunoprecipitation (IP)	Assay-dependent
Immunogen	A peptide corresponding to amino acids 225 to 239 of human Smac /DIABLO.	Western Blot (WB)	1 µg/mL
		Immunocytochemistry (ICC/IF)	5 µg/mL
Conjugate	Unconjugated	<div>* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.</div>	
Form	Liquid		
Concentration	1 mg/mL		
Purification	Antigen affinity chromatography		
Storage buffer	PBS		
Contains	0.02% sodium azide		
Storage Conditions	Maintain refrigerated at 2-8°C for up to 3 months. For long term storage store at -20°C		

**Product specific information**

In Western blot applications, this antibody detects a band at ~25kDa. A suggested positive control is human heart tissue lysate. PA5-19974 can be used with blocking peptide PEP-0099.

**Background/Target Information**

DIABLO (smac), a mitochondrial protein, activates various forms of apoptosis. This activation may be due to the neutralization of one or more members of the IAP family of apoptosis inhibitory proteins. Smac exits the mitochondria and enter the cytosol during certain apoptosis triggered events. Mitochondrial-mediated apoptosis is important in animal development and tissue homeostasis, with alterations resulting in a range of malignant disorders. Upon apoptotic stimuli, the mitochondrial proteins cytochrome c and Smac/DIABLO are released into the cytosol. The release of these proteins, however, occurs via different mechanisms. Smac/DIABLO has also been found to play a key role in regulating the sensitization of cancer cells to apoptosis (both immune and drug-induced).

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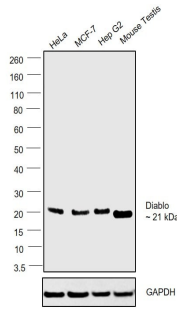
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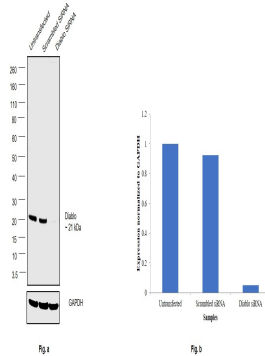
DIABLO Antibody (PA5-19974) in WB

Western blot was performed using Anti-DIABLO Polyclonal Antibody(Product # PA5-19974) and a 21 kDa band corresponding to DIABLO was observed across the cell lines and tissues tested. Whole cell extracts (30 µg lysate) of HeLa (Lane 1), MCF-7 (Lane 2), Hep G2 (Lane 3) and Mouse Testis (Lane 4) were electrophoresed using NuPAGE™ 4-12% Bis-Tris Protein Gel (Product # NP0321BOX). Resolved proteins were then transferred onto a Nitrocellulose membrane (Product # LC2001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (1 µg/mL) and detected by chemiluminescence with Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036,1:4,000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005).



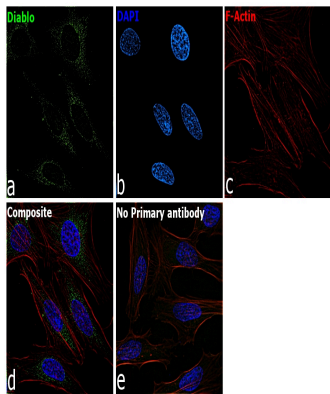
DIABLO Antibody (PA5-19974)

Antibody specificity was demonstrated by siRNA mediated knockdown of target protein. HeLa cells were transfected with DIABLO siRNA and decrease in signal intensity was observed in Western Blot application using Anti-DIABLO Polyclonal Antibody (Product # PA5-19974). {KD}



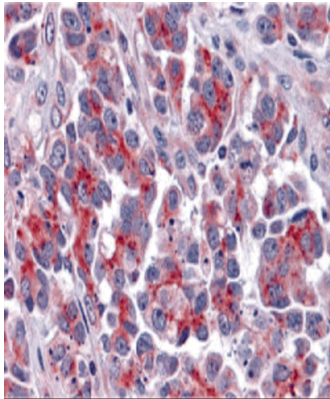
DIABLO Antibody (PA5-19974) in ICC/IF

Immunofluorescence analysis of DIABLO was performed using 70% confluent log phase HeLa cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 10 minutes, and blocked with 2% BSA for 45 minutes at room temperature. The cells were labeled with DIABLO Polyclonal Antibody (Product # PA5-19974) at 5 µg/mL in 0.1% BSA, incubated at 4 degree celsius overnight and then labeled with Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, Alexa Fluor® 488 conjugate (Product # A27034), (1:2000 dilution), for 45 minutes at room temperature (Panel a: Green). Nuclei (Panel b:Blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: Red) was stained with Rhodamine Phalloidin (Product # R415, 1:300 dilution). Panel d represents the merged image showing Mitochondrial localization. Panel e represents control HeLa cells with no primary antibody to assess background. The images were captured at 60X magnification.



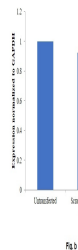
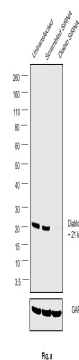
DIABLO Antibody (PA5-19974) in IHC (P)

Immunohistochemical analysis of paraffin-embedded Human Ovary tissue using DIABLO Polyclonal Antibody (Product # PA5-19974) at 5 µg/mL. Tissue was fixed with formaldehyde and blocked with 0.1 serum for 1 h at RT; antigen retrieval was by heat mediation with a citrate buffer (pH6). Samples were incubated with primary antibody overnight at 4C. A goat anti-rabbit IgG H&L (HRP) at 1/250 was used as secondary. Counter stained with Hematoxylin.



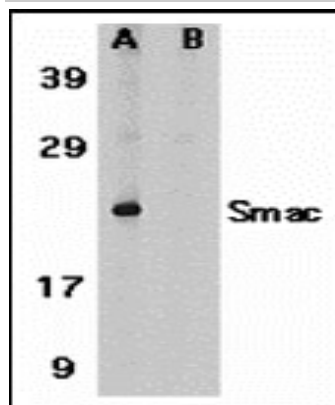
## DIABLO Antibody (PA5-19974) in WB

Knockdown of DIABLO was achieved by transfecting HeLa with DIABLO specific siRNAs (Silencer® select Product # S32187, S32188). Western blot analysis (Fig. a) was performed using Whole cell extracts from the DIABLO knockdown cells (lane 3), non-targeting scrambled siRNA transfected cells (lane 2) and untransfected cells (lane 1). The blot was probed with DIABLO Polyclonal Antibody (Product # PA5-19974, at a concentration of 1 µg/mL) and Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:4,000 dilution). Densitometric analysis of this western blot is shown in histogram (Fig. b). Decrease in signal upon siRNA mediated knock down confirms that antibody is specific to DIABLO.



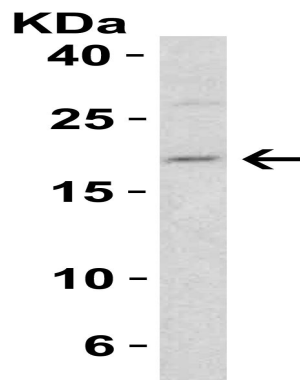
## DIABLO Antibody (PA5-19974) in WB

Western Blot Validation in Human Heart Tissue Lysate. Loading: 15 µg of lysates per lane. Antibodies: DIABLO Polyclonal Antibody (Product # PA5-19974) (1 µg/mL), 1h incubation at RT in 0.05 NFDN/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10,000 dilution. (A) Without blocking peptide (B) With blocking peptide



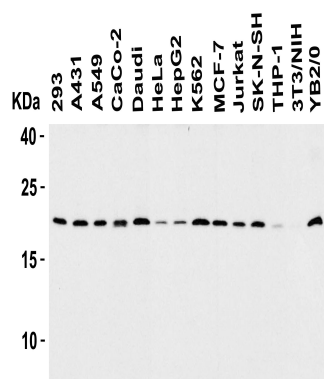
## DIABLO Antibody (PA5-19974) in WB

Western Blot Validation in Mouse 3T3/NIH Cells. Loading: 15 µg of lysate. Antibodies: DIABLO Polyclonal Antibody (Product # PA5-19974) (1 µg/mL), 1h incubation at RT in 0.05 NFDN/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10,000 dilution.



## DIABLO Antibody (PA5-19974) in WB

Western Blot Validation in Human, Mouse and Rat Cell Lines. Loading: 15 µg of lysates per lane. Antibodies: DIABLO Polyclonal Antibody (Product # PA5-19974) (1 µg/mL), 1h incubation at RT in 0.05 NFM/TBST. Secondary: Goat anti-rabbit IgG HRP conjugate at 1:10,000 dilution.



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